# *Beauveria bassiana* Horizontal Infection Between Cadavers and Adults of the Colorado Potato Beetle, *Leptinotarsa decemlineata* (Say)

ELLEN KLINGER,[1] ELEANOR GRODEN,[2] AND FRANCIS DRUMMOND[2]

Environ. Entomol. 35(4): 992–1000 (2006)

**ABSTRACT** The behavior of adult Colorado potato beetles (*Leptinotarsa decemlineata*) in the presence of *Beauveria bassiana* sporulating on cadavers was studied to determine the likelihood of disease infection as beetles emerge from the soil and colonize host plants. In 2001, the movement of single adult Colorado potato beetles was monitored in arenas containing infective cadavers in differing spatial patterns between four potato plants. In 2002, a similar design was used, but was under the more natural conditions of a potato field. In both experiments, direction, time, and directness of beetle travel was not significantly affected by the presence or absence of infective cadavers, showing no avoidance of these cadavers by adult Colorado potato beetles. The likelihood that emerging adults would contact infective cadavers on the soil surface was quantified at different cadaver densities. A curvilinear relationship ($y = 4.8313x^{0.4459}$) best describes the frequency of encounters of adults as a factor of increasing density of cadavers on the soil surface. Mortality of adults and production of conidia on cadavers after encounters with increasing densities of infective cadavers had similar relationships that show the decreased susceptibility of adults to *B. bassiana* compared with prepupae. These relationships were used to model horizontal infection to emerging adults in the summer months, subsequent to a conidial spray targeted at larvae. The model indicates that horizontal infection of adults may potentially be significant (30–70% of horizontal infection), and that simulated early sprays targeted at first-instar larvae resulted in the maximum levels of predicted horizontal infection.

**KEY WORDS** *Beauveria bassiana*, Colorado potato beetle, horizontal infection, avoidance, adult

*Beauveria bassiana* (Balsamo) Vuillemin is a fungal pathogen of many insect species across a number of orders (Tanada and Kaya 1993). Its development as a biological control agent has been of considerable interest as problems with insecticide resistance and insecticide residue become limiting factors in crop production (Huang et al. 1995, Stewart et al. 1997, Olson et al. 2000, Zhao et al. 2000, Pimentel 2005). Applications of *B. bassiana* have been used to successfully control the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Campbell et al. 1985, Hajek et al. 1987, Anderson et al. 1988). However, because *B. bassiana* conidia are degraded by UV light and dispersed by rainfall, and because temperature and humidity can impact infection rates, direct effects of conidial sprays can be short lived and varied (Walstead et al. 1970, Daoust and Pereira 1986, Inglis et al. 2000). Once primary infection of *B. bassiana* occurs within a host population, secondary or horizontal infection can increase the proportion of the population that becomes infected with disease. Horizontal infection has been documented in many insect/entomopathogen systems (Brown and Nordin 1982, Thomas et al. 1995, Long et al. 1999, Furlong and Pell 2001). For healthy Colorado potato beetle individuals, horizontal infection can occur when prepupae and adults moving across soil surfaces contact conidiating *B. bassiana* present on beetle cadavers (Long et al. 2000a). Long et al. (2000a) found that Colorado potato beetle late-stage larvae or prepupae were more likely to come in contact with cadavers with conidiating *B. bassiana* (infective cadavers) on the soil surface as cadaver density increased, and subsequent mortality and infection increased with increasing cadaver density. *B. bassiana* conidial sprays that are targeted at Colorado potato beetle larval stages usually produce infective cadavers on the soil surface that can remain viable for over a month (Fernandez 2000) and coincide with the emergence of new adults from pupation sites in the soil (Long et al. 2000b). The Colorado potato beetle adult is not as susceptible to *B. bassiana* as the larval stages (Fargues 1972), but we hypothesize that they may acquire significant infections from contact with these infective cadavers.

The purpose of this study was to determine the probability that adult Colorado potato beetles emerging from the soil and subsequently moving to colonize potato plants will encounter *B. bassiana* infective cadavers. Experiments were conducted to (1) determine if adult Colorado potato beetles avoid contact with cadavers and (2) quantify the relationships be-

[1] Corresponding author: USDA Bee Biology and Systematics Laboratory, 1410 N. 800 E., N. Logan, UT 84341 (e-mail:eklinger@biology.usu.edu).

[2] Department of Biological Sciences, University of Maine, Orono, ME 04469.

tween infective cadaver density, contact, and infection of newly emerged adult Colorado potato beetles. An existing horizontal infection model (Long et al. 2000b) was modified to estimate the significance of horizontal transmission to Colorado potato beetle adults to the population dynamics of this pest.

## Materials and Methods

### Insects and Foliage

Colorado potato beetle adults were reared from laboratory colonies that were supplemented annually with field-collected adults from the University of Maine's Rogers Farm in Stillwater, ME. Potato fields used for beetle collections received seven total weekly sprays of the copper hydroxide fungicide, Champ Formula 2 (Agtrol Chemical Products, Houston, TX) for late blight (caused by *Phytophthora infestans*) control, and no other pesticides were applied to the fields. All collected adult beetles were maintained in 300-ml paper cups held at 25 ± 1°C and a 16:8 light-dark cycle and fed fresh greenhouse grown potato foliage daily (*Solanum tuberosum* L.), primarily variety Kennebec, with the exception of 'Delta Gold' for density experiments. To rear beetles for experiments, egg masses were collected daily from culture cups. On hatch, ≈20 larvae were placed in 300-ml paper cups and raised under the same conditions as adults. When larvae entered the prepupal stage, they were placed in pupation containers (petri dishes lined with moist paper toweling), held in the dark at 25 ± 1°C, and checked daily for adult emergence.

### Cadaver Production

*Beauveria bassiana* strain GHA was obtained as dried conidia from Mycotech, now Laverlam International (Butte, MT), and plated on Sabouraud dextrose agar (SDA; Difco Laboratories, Detroit, MI). Colonies were subcultured once on SDA, and for each experiment, one of these single, axenic subcultures (grown for 10 d at 25 ± 1°C and stored at 4 ± 1°C for ≤60 d) were used. Conidia were harvested from these plates with a sterile loop and suspended in an aqueous solution of 0.1% Tween 20 (JT Baker, Phillipsburg, NJ). Concentrations of conidia were determined using a hemocytometer, and aqueous solutions were diluted as necessary to achieve $2 \times 10^7$ conidia/ml in 0.1% Tween 20. To produce cadavers for these experiments, either adults or late second-instar larvae from the laboratory culture were sprayed with this *B. bassiana* solution using a Burkhard Computerized Spray Apparatus (Hertfordshire, United Kingdom) at 55.2 kPa at a diaphragm setting of six. Two plates of SDA were sprayed to obtain accurate rates of conidial density and viability at the time of each spray. In all experiments, densities ranged from 695 to 1,333 conidia/$mm^2$, and ≥98% of the conidia had formed germ tubes after 18 h at 25 ± 1°C, indicating viability. Sprayed beetles were held in a sealed plastic container (38 by 26 by 13.5 cm) lined with moist paper toweling, and fed fresh potato foliage on a daily basis. Once beetles had died and the fungus had produced conidia, infective cadavers were stored in a petri dish lined with cheesecloth on top of moistened filter paper. Cadavers were stored at 4 ± 1°C and used within 3 d of initial conidia production. At the end of each day of observations, one or more of the cadavers used in the experimental arena were sampled for conidia viability by touching a sterile needle to the side of the cadaver and streaking onto an SDA plate. Viability was determined microscopically by the presence of a germ tube on conidia after 18 h at 25 ± 1°C.

### Avoidance of Adult Colorado Potato Beetles to Infective Cadavers

In 2001, beetle behavior was studied in a wooden arena designed to simulate a field setting. The same behavior was studied in the summer of 2002 in a similar experiment set in the more natural conditions of a potato field.

**2001.** In June 2001, wooden arenas were constructed with an 80 by 65-$cm^2$ plywood bottom and 14-cm-high sides, elevated on 30-cm legs such that the box sat off of the ground to accommodate recessed potted potato plants. Four 19.5-cm-diameter holes were cut into the plywood bottom of the box, and 19-cm-diameter plastic plant pots were secured in these holes with silicon caulking. Metal flashing was attached to the inner sides of the box with Liquid Nails (Macco, Cleveland, OH) to prevent beetles from climbing the surface (see Klinger 2003, Fig. 3.1 for diagram of arena).

To conduct the experiment, three arenas were placed out of doors at the University of Maine, Orono campus. A layer of sieved (1-$cm^2$ mesh) Caribou gravelly loam collected from the University of Maine's Aroostook Farm in Presque Isle, ME, was placed in the bottom of the arena to a depth of ≈5 cm to create an even surface with the top of the recessed pots and not restrict beetle movement. The soil was moistened with water from a spray bottle before each assay. North-south oriented pots were spaced at a distance of 30.5 cm to mimic distances between plants in a conventional potato field. The center of each plant was located 30 cm from the center of the arena, which is the average distance that prepupae travel before burrowing into soil in the field (Long et al. 2000b). Every 3–5 d during the assay runs, new greenhouse-grown potted potato plants were placed in the arena. A 5-$cm^2$ grid made of cotton string (180 squares total) was superimposed and suspended over the top of the arena to aid in quantifying beetle movements.

Individual beetle movement was monitored within the arenas relative to the potted potato plants and the proximity or absence of infective cadavers. Temperature and relative humidity were monitored using a HOBO Pro Series Datalogger (Onset Computer Company, Bourne, MA). For each trial, one beetle was placed just under the soil in the center of the arena and was observed by either a human observer or a video camera (Sony Video 8XR Vision Handycam; Sony, Tokyo, Japan). In the case of human observation, the observer remained directly overhead of the beetle as it traveled, and, using a stopwatch and a grid diagram, recorded the path and time for the beetle to colonize a potato plant. Data recorded were (1) time to reach a plant; (2) time spent in each square; and (3) which of the four plants the beetle colonized. A beetle was considered to have colonized a plant when its entire body was off the soil and in contact with the plant. Beetles that did not move for 20 min or left the arena by climbing up the side were removed and were not used for the analyses of total time for plant colonization or specific plant colonized. Because the video camera was a stationary observer and thus unable to stay directly overhead of the beetle, recorded observations were corrected for parallax error by resetting the distance between grid lines to the 5-$cm^2$ spacing (this method was determined preexperiment to be as accurate as a human observer). The video camera recorded the trial for at least 20 min, after which, if no movement was observed, a new trial was begun.

Arena positions were rotated between individual beetle trials so that any orientation to a cardinal direction could be noted. After a beetle had colonized a plant, it was put in an individual 60 by 15-mm petri dish with moistened filter paper, fed fresh greenhouse-grown potato foliage, and maintained at 25 ± 1°C and a 16:8 light-dark cycle for 21 d. At 24-h intervals, each beetle was checked for mortality, and dead beetles were placed in a petri dish at 100% RH and monitored for signs of *B. bassiana,* which was indicated by the observance of *B. bassiana* conidia on the outside of the insect.

Control runs of the experiment consisted of the arena with the potato plants and no beetle cadavers. Infective cadaver treatments consisted of cadavers with conidiating fungus arranged in U-shape patterns surrounding the beetle release point, ≤7.5 cm away from the release point. This U-shape pattern resulted in only one possible path for a beetle to travel toward a plant and traverse only cadaver-free grid squares (see Klinger 2003, Fig. 3.2 for diagram). The opening in the pattern was randomly rotated between assay runs to avoid potential bias from beetles traveling in one direction.

**2002.** A similar design was used for a field study in 2002 as was used for the outdoor arena study in 2001. However, in 2002, arenas consisted of a 1.2 by 0.9-m bottomless frame that was placed over four plants in two adjacent rows of the same potato plot at the University of Maine's Rogers Farm (Stillwater, ME) as described in the methods for beetle collection. The frames accommodated a similar but larger grid as the 2001 arenas with 391 5-$cm^2$ squares per frame. Assay runs were performed and environmental conditions were monitored as described in 2001; however, all observations were made by human observers in 2002, and beetles were not placed under the soil at the start position because the soil was too compact.

**Data Analysis.** A $\chi^2$ analysis was used to determine whether the choice of plants by beetles was random. The probability of a beetle randomly passing through a cadaver filled square as it left the center was calculated, based on the number of squares in each trial that contained infective cadavers, and the total number of squares surrounding the release point. $\chi^2$ analysis was used to determine if beetle travel through these squares was random. One-way analyses of covariances (ANCOVAs; JMP 2001) were performed to determine if there were any significant differences between the main effects of beetle age, temperature, relative humidity (covariates), and infective cadaver treatments in relation to total time for beetles to colonize plants and in relation to total number of grid squares the beetle passed through to colonize a plant (a measure of directness). In all ANCOVAs, the dependant variable data were log-transformed to produce homoscedacity. One-way analysis of variance (ANOVA) was used to assess relationships between the average time a beetle spent in a square and *Beauveria* treatment. Nominal logistic regression analysis (JMP 2001) was used to determine if there was any significant effect of *Beauveria* treatment, temperature, relative humidity, or beetle age on the occurrence (likelihood) of a beetle not moving and subsequently being eliminated from a trial. All analyses were conducted at an alpha level of 0.05.

### Infective Cadaver Density and Infection of Adult Colorado Potato Beetles

To study the relationship between cadaver density on the soil surface and adult beetle infection, the same arenas used in the behavioral assays in 2001 were used; however, these experiments were performed indoors in a room absent of sunlight cues. *B. bassiana* infective larval cadavers were placed on the soil surface at densities of 0, 0.0173, 0.0289, 0.0606, and 0.2352 cadavers/$cm^2$ within a 693-$cm^2$ area surrounding the beetle release point. An adult beetle was placed in the center of the arena and was allowed to colonize one of four potato plants. The numbers of cadavers encountered by beetles were recorded, and each cadaver encounter was given a numerical rating: (1) contact with a cadaver that lasted <1 s and involved either a leg or antennae touching the cadaver only, (2) contact with a cadaver on the underside of the live beetle's body for 1–2 s, and (3) contact lasting >2 s and involved sustained contact with some part of the beetle's head, thorax, or abdomen. Control treatments were noninfective larval cadavers placed in the same densities as the infective cadaver treatments. These noninfective cadavers were put together under the category of zero cadavers per square centimeter for analyses of mortality and infection but were included for each density when analyzing amount and durations of contact. For the contact analysis, a zero density of infective cadavers was preset to equal zero contacts. Duration of encounter, temperature, and relative humidity were recorded. All beetles were maintained in 60 by 15-mm petri dishes at same conditions as beetles in avoidance experiment and held for 2 wk to assess death and fungal infection.

ANCOVAs (JMP 2001) were used to determine the significance of age (covariate), density, and treatments in relationship to the total number of cadaver contacts by a beetle during a trial. Linear regression (SigmaPlot 1998) was used to determine relationships between cadaver density and the number of cadaver encounters. Nominal logistic regression, Wald's test criterion (JMP 2001), was used to determine the significance of beetle age, density, and treatment to subsequent adult mortality and fungal infection. Linear regression (form: $Y = aX^b$) was also used to determine relationships between cadaver density and mean proportion mortality and fungal infection. One-way ANCOVAs were used to determine the significance of main factors age, temperature, relative humidity (covariates), and *Beauveria* treatment affecting time to plant colonization, as well as the average rating of contact (referred to as the quality of contact) in any given trial.

### Modeling Horizontal Infection of Newly Emerged Summer Colorado Potato Beetle Adults

A simulation model of both larval primary infection from sprayed conidia and prepupal secondary or horizontal infection from contact with infective cadavers was previously constructed (Long et al. 2000b) and validated (Long et al. 2000b, Joergensen 2000). In this temperature-dependent model, each insect stage is simulated by using a time-varying distributed delay (TVDD) (Carruthers et al. 1988). This computer simulation model incorporates both the levels of *B. bassiana* causing larval mortality and the time to death caused by mycosis. The spatial distribution of larvae (larval densities between plants within a field, the spatial distributions of cadaver density around plants, and the distribution of distances that prepupae move from a plant to the site of burrowing before pupation) is also incorporated into the model. The aspect of the horizontal infection process that we used this model to assess occurs when prepupae or adults encounter cadavers with conidiating fungus on the soil surface and the probability of becoming infected is determined by the contact of cadavers by wandering prepupae or adults. Data from our avoidance and density experiments with adult beetles were incorporated into this model to simulate horizontal infection of *B. bassiana* from infective cadavers to newly emerged summer adults.

We incorporated horizontal infection of prepupae and adults emerging from their pupation sites into the model with the following assumptions: (1) the period that cadavers are infective is 400 DD (base 10°C, Fernandez 2000, Long et al. 2000b) and not affected by the detrimental effects of rainfall or sunlight because the potato canopy is closed over late in the growing season at the time when horizontal infection would take place (Fernandez 2000, M. Furlong and E. Groden, unpublished data); (2) Colorado potato beetle adults search for plants in the same areas they searched for a pupation site when prepupae; (3) there is no avoidance behavior toward infective cadavers by Colorado potato beetle adults; (4) the relationship between adult encounters with infective cadavers as they search for potato plants and subsequent likelihood of infection can be described by the regression relationships developed through our experiments described above; and (5) sublethal infection does not play an important role in horizontal infection. Long et al. (2000b) discuss other assumptions underlying the structure of the model.

The specific horizontal infection model for adults incorporates the encounter rate with cadavers, spatial distribution of infective cadavers on the soil surface, and the movement of adults to their respective host plants (as modified from Long et al. 2000b). The general submodel without any intrinsic spatial structure is

$$HT_t = 1.6544 \times C_t^{0.4811} \text{ (parameter estimates from data plotted in Fig. 2)} \quad [1]$$

where $HT_t$ = proportion of adults becoming infected at time t (dt = 2.4 h) and $C_t$ = density (per $cm^2$ of soil surface) of infective cadavers at time t.

As described by Long et al. (2000b), the horizontal infection submodel becomes modified when the spatial distribution of the cadavers and the Colorado potato beetle host stage is taken into account. The spatial pattern of larvae on plants within a potato field is well described by a negative binomial distribution (Mena-Covarrubias et al. 1996). This spatial pattern determines the pattern of cadavers and thus the probability of prepupae and adults contacting them. Therefore, a frequency distribution of larvae is used to calculate infection for each plant using equation 2 iteratively to calculate a field-level infection.

$$HT_t = \Sigma 1.6544\,(J_i C_t A_i^{-1})^{0.4811} (A_i/625)\,(B_i P_i) \quad [2]$$

where $HT_t$ = total proportion horizontal infection of newly emerged Colorado potato beetle adults at time t, $\Sigma$ = sum across all concentric rings (from one to i) of 2 cm width around the emerging beetle out to its host plant, $J_i$ = spatial pattern (Johnson distribution) (Slifker and Shapiro 1980) of cadavers in region i, $C_t$ = infective cadaver density at time t, $A_i$ = area of region i ($cm^2$), $B_i$ = proportion of total adults available to be infected in region i, and $P_{i,t}$ = total number of adults in region i at time t.

To model horizontal transmission to adults in a potato field, we used the same methodology as Long et al. (2000b). The effect of environmental conditions on adult movement rate was not taken into account in the model.

Simulations were conducted using air temperature data sets measured in potato fields at the University of Maine Aroostook Potato research Farm (Presque Isle, ME) to determine the range of expected horizontal infection given an unusually cool growing season (1993), an unusually warm growing season (1995), and one of the warmest growing seasons for the past 50 yr (2002). These model runs were also conducted to simulate a range of different timings of sprays that initiate primary infection (sprays applied at early first-instar through peak fourth-instar incidence). Graphical analysis of primary infection, horizontal infection of prepupae, and horizontal infection of adults were used to interpret the effect that inclusion of horizontal infection of adults has on the epidemiology of *B. bassiana.*

## Results

### Avoidance of Adult Colorado Potato Beetles to Infective Cadavers

Three hundred six beetles were tested in both 2001 and 2002. Of these, 50 beetles had to be eliminated from total time to colonize plant and plant colonized analyses for not moving within the allotted time or for climbing up the side of the arena. Of the remaining 256 usable beetle trials, 95 were in 2001 and 161 were in 2002.

### Plant Colonization

There was no effect of *Beauveria* treatment on the number of beetles that were eliminated from the trial for not moving within the time allotted (Wald $\chi^2_{(1,305)} = 2.84$, $P = 0.09$). In 2001 and 2002, neither beetles in the control treatment nor beetles in infective cadaver treatments colonized one of four plants at an observed frequency significantly different from the expected frequency characterized by random cardinal direction ($\chi^2 = 7.00$, 3 df, $P < 0.10$; $\chi^2 = 4.06$, 3 df, $P < 0.50$, for 2001 and 2002, respectively, for control treatments; $\chi^2 = 0.08$, 3 df, $P < 0.99$; $\chi^2 = 1.54$, 3 df, $P < 0.75$, for 2001 and 2002, respectively, for infective cadaver treatments), indicating that beetles did not move toward a particular direction during our experiments.

In both 2001 and 2002, beetles did not show avoidance of infective cadavers. In 2001, the hypothesized probability of a beetle passing through a random grid square surrounding the release point was 0.10. Beetles did not deviate from this expected frequency for the infective cadaver treatment ($\chi^2 = 1.27$, 1 df, $P < 0.50$). In 2002, the expected probability of a beetle passing though a random grid square surrounding the release point was 0.08. Beetles in this experiment also did not deviate from this expectation ($\chi^2 = 0.08$, 1 df, $P < 0.90$).

Of 112 beetles that were exposed to cadavers in both 2001 and 2002, 21 had one type of direct contact with a cadaver during the study. Of these beetles, six subsequently died after trial, but of these six, only one produced *B. bassiana.* Of the 91 that did not contact a cadaver, 14 subsequently died, and none showed signs of *B. bassiana* growth.

### Beetle Activity

Analysis of environmental impacts on beetle movement revealed significant effects of relative humidity on (1) the distance traveled in 2001 only ($F_{(1,94)} = 5.76$, $P = 0.018$) and (2) the time period it took to travel from the point of emergence from the soil to the place of colonizing their host plant, in 2001 ($F_{(1,94)} = 12.11$, $P = 0.0008$) and 2002 ($F_{(1,160)} = 4.30$, $P = 0.040$), with longer times and less direct travel associated with higher humidity. Presence of infective cadavers in a trial did not alter the average amount of time a beetle spent in a square in either year ($F_{(1,253)} = 0.098$, $P = 0.09$; two beetle trials had to be eliminated from this analysis because of a camera incident).

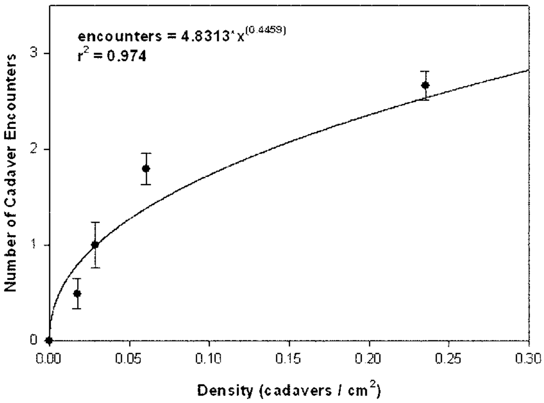


**Fig. 1.** Relationship between the number of cadaver encounters by adult Colorado potato beetles (square root transformed) and density of second-instar cadavers in laboratory bioassays. Figures depict mean ± SE.

### Infective Cadaver Density and Infection of Adult Colorado Potato Beetles

Sixty-seven beetles were used for the cadaver density studies. For densities of 0 ($n = 12$), 0.0173 ($n = 5$), 0.0289 ($n = 11$), 0.0606 ($n = 28$), and 0.2352 ($n = 11$) cadavers/cm$^2$, encounters of adult beetles with infective cadavers ranged between 0.25 and 6.25 encounters per individual. The frequency an adult beetle encountered an infective cadaver on the soil surface before plant colonization increased as density of cadavers increased ($F_{(1,66)} = 27.58$, $P < 0.0001$) and is described by the linear regression between the mean number of encounters and the density of cadavers in the form $Y = aX^b$ (Fig. 1). Quality of contact was directly proportional to cadaver density ($F_{(1,66)} = 7.67$, $P = 0.007$; Table 1). There was no relationship between either beetle mortality or infection and increasing quality of contact. Mortality of adults also increased as cadaver density increased ($F_{(1,66)} = 4.92$, $P = 0.026$) as did infection ($F_{(1,66)} = 4.41$, $P = 0.039$). These relationships were modeled by regression of the average proportion mortality and average proportion

**Table 1. Average rating (±SE) of the degree or quality of contact between adult Colorado potato beetles and infective cadavers over a range of cadaver densities**

| Density (cadavers/cm$^2$) | Average rating |
|---|---|
| 0.0173 | 0.12 ± 0.33 |
| 0.0289 | 0.82 ± 0.57 |
| 0.0606 | 1.40 ± 0.69 |
| 0.2352 | 1.23 ± 0.23 |

Ratings range from 0 to 3.

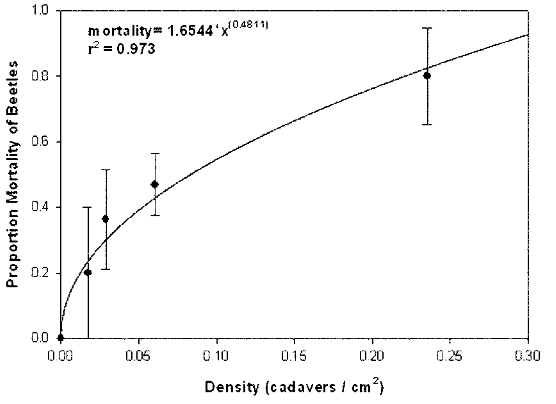


**Fig. 2.** Relationship between density of *B. bassiana* infective second-instar Colorado potato beetle cadavers and mortality of adult Colorado potato beetles. Figures depict mean ± SE.

of beetles exhibiting fungal infection by the density of cadavers on the soil surface in the form $Y = aX^b$ (Figs. 2 and 3).

Presence of infective cadavers did not impact the total time it took for a beetle to colonize a plant ($F_{(1,66)} = 0.98$, $P = 0.33$), but total time was affected by age ($F_{(1,66)} = 9.12$, $P = 0.004$), with older beetles taking more time to colonize plants. Increased densities of cadavers on the soil surface did not result in increased time to colonize plants ($F_{(1,66)} = 2.64$, $P = 0.10$).

## Modeling Horizontal Infection of Newly Emerged Summer Colorado Potato Beetle Adults

Predictions of the incidence of healthy and infected Colorado potato beetle larvae, infective cadavers, and emerging adults from model runs using observed egg incidence in the field and weather data from 1995 Presque Isle, ME, indicated considerable opportunity for horizontal infection to adults. Over the 3 yr for which simulations were run, horizontal infection increased 30–70% when adults were included as susceptible hosts in the model compared when only prepupae were available for horizontal disease transmission. Primary infection was maximized for single applications targeted at peak second-instar occurrence, independent of year (Fig. 4A). Maximum primary infection occurs for multiple applications when two applications were made first during peak first-instar occurrence and again at peak second-instar occurrence (Fig. 4A). However, horizontal infection is maximized when a single conidial application was made during peak first-instar occurrence or when two were made bracketing the peak first-instar occurrence (Fig. 4B).

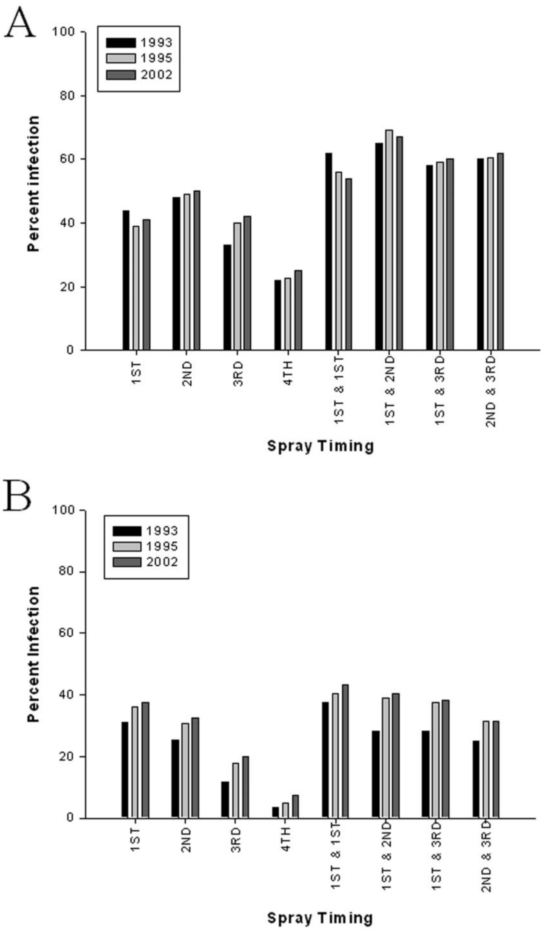


**Fig. 4.** Simulation results indicating relationship between the timing of the application of *B. bassiana* conidia in 1993, 1995, and 2002 and (A) the resulting primary infection of Colorado potato beetle populations (expressed as a percent of individuals infected relative to total population density) and (B) horizontal infection (expressed as percent infection of the adults that escaped primary infection).

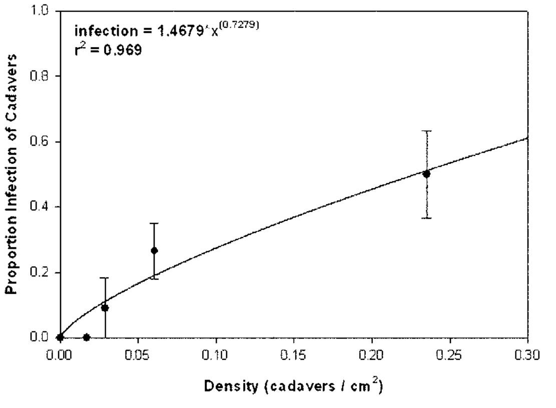


**Fig. 3.** Relationship between density of *B. bassiana* infective second-instar Colorado potato beetle cadavers and the proportion infection of adult Colorado potato beetles. Figures depict mean ± SE.

## Discussion

Insect avoidance of pathogenic fungi has been described in some systems. Termites show avoidance of cadavers with conidiating fungus but do not avoid live diseased individuals (Kramm et al. 1982). Villani et al. (1994) found that Japanese beetle grubs burrowing through soil actively avoid areas where *Metarhizum anisopliae* conidia had been added. However, Lord (2001) found that *Cephalonomia tarsalis,* a parasitoid of sawtoothed grain beetles (*Oryzaephilus surinamensis*), do not distinguish between *B. bassiana* infected and noninfected beetles, although these parasitoids are susceptible to the disease. These wasps were also found to enter conidia positive environments at the same frequency as clean environments. Colorado potato beetle adults also lack an avoidance to *B. bassiana* infective cadavers. In both our controlled arena and field experiments, the adults did not show any evidence of avoiding infective cadavers, the cadavers had no impact on orientation, and no avoidance of areas with cadavers present or with the cadavers themselves.

Although relative humidity was the only abiotic factor that was significantly correlated with beetle movement, its impact was minor in this study. It has been reported that decreases in humidity result in increased movement of insects (Fraenkel and Gunn 1940). In this study, similarly, adult movement was slightly higher at lower relative humidities.

We showed that *B. bassiana* conidia can be transmitted to emerging adult beetles that contact an infective cadaver, and the frequency of encounters with cadavers increases as cadaver density increases in a curvilinear fashion. This relationship reflects the fundamental transmission dynamics first developed by Kermack and McKendrick (1927), which is the basis for much epizootiological modeling: $dS/dt = -\lambda_t S_t$, where S = susceptible hosts, t = time, and $\lambda$ = force of infection at time t. It has since been modified to incorporate more complex dynamics (Anderson 1981, Brown and Nordin 1982, Kelly-Tunis et al. 1995, Thomas et al. 1995, Long et al. 2000b). Our results differ from that described by Long et al. (2000a) for the relationship between the density of *B. bassiana* infective cadavers and encounters with burrowing prepupae. In this study, adults came in contact with cadavers at a higher rate than was found for prepupae (prepupae averaged between 0.01 and 2.25 encounters per individual). Their prepupal studies also described a linear relationship between number of encounters by prepupae and cadaver density, whereas we found a curvilinear relationship. This difference could come from lower numbers of encounters in the prepupal study (i.e., possibly capturing the initial portion of the curvilinear relationship and not being able to detect the curvilinear nature of the relationship over the range of cadaver densities). On general observation, adult Colorado potato beetles are more mobile than prepupae and are more likely to have multiple encounters even at lower densities of cadavers. The observed curvilinear relationships between adult Colorado potato beetles encountering cadavers and subsequent mortality and signs of fungal infection are similar to the findings of Long et al. (2000a), with, on average, prepupae sporulating 36.8% more than adult beetles. This is most likely caused by the greater susceptibility of larvae to *B. bassiana* than adults (Fargues 1972).

Simulation results indicated that there is ample opportunity for adult beetles to encounter infective cadavers in the field, primarily because of the long persistence of infective cadavers (not unlike that observed in the field; Fernandez 2000). Because of this persistence, summer adults will tend to emerge and move across the soil when the incidence of infective cadavers is at its peak. Modeling horizontal infection of prepupae and adults suggested that the inclusion of adults can increase overall infection levels, depending on the timing of the application and the rate of larval development. The growing season air temperature regime affects healthy larval development times, time to death of infected larvae, the duration of infective cadavers, and the time of occurrence of prepupae and adults. However, even though we used temperature regimens from 3 very different yr, our simulations suggest that the resulting levels of horizontal infection vary only moderately between years, up to 15%. More variation in horizontal infection was attributable to the timing of primary infection. This is caused by the developmental rate of healthy individuals relative to the time to death of infected larvae and the time to production of fungal conidia on cadavers. When sprays are applied later, relative to the incidence of small larvae, a greater proportion of the adult population can escape encounters with cadavers if they emerge before cadavers become infective (there is a temperature-dependent lag between host death and conidial production on cadavers). Some caution must be exercised in our conclusions because only portions of our simulation model have been validated in the field (primary infection, Joergensen 2000; horizontal infection of prepupae, Long et al. 2000b), and no validation of adult populations has been performed. However, because the mechanism of horizontal infection is very similar between prepupae and adult beetles (encounters on the soil surface with infective cadavers), we believe our conclusion, based on simulation modeling, that horizontal infection of emerging adults can be an important dynamic in the field is reasonable.

Additional factors not included in this study may play a role in the level of actual horizontal infection in the field. Long et al. (2000a) found horizontal infection of prepupae increased with lower temperatures and that temperature could not explain differences in encounters with cadavers, time to burrow, and depth of burrowing, suggesting that temperature has a greater impact on fungal growth than it does on beetle behavior. In fact, temperature is thought to be the most important external factor in determining insect susceptibility and the multiplication of a microbial pathogen within its host (Watanabe 1987). Humidity has been shown here to affect beetle movements, but weakly, and it may play a more important role in the incidence of *B. bassiana* infection in Colorado potato beetle populations. Solar radiation, rainfall, and reduction of scavengers in the field by other arthropod scavengers may play a role in the level of disease infection in the field (Fernandez 2000, Joergensen 2000, Coluzzi 2005, M. Furlong and E. Groden unpublished data). These other factors, as well as field validation of our model, should receive further study to better predict expected disease outcomes in the field environment.

## Acknowledgments

We thank T. Timms, P. Bolin, S. Cambron, and N. Delisle for technical assistance and R. James for useful comments on the manuscript. The Maine Agricultural and Forest Experiment Station (MAFES) and USDA-NRI Grant 98-35316-6472 funded this work. This is MAFES publication 2873.

## References Cited

**Anderson, R. M. 1981.** Population ecology of infectious disease agents, pp. 318–355. *In* R. M. May (ed.), Theoretical ecology. Blackwell Scientific, Boston, MA.

**Anderson, T. E., D. W. Roberts, and R. S. Soper. 1988.** Use of *Beauveria bassiana* for suppression of the Colorado potato beetle in New York state (Coleoptera: Chrysomelidae). Environ. Entomol. 17: 140–145.

**Brown, G. C., and G. L. Nordin. 1982.** An epizootic model of an insect-fungal pathogen system. Bull. Math. Biol. 44: 731–739.

**Campbell, R. K., T. E. Anderson, M. Semel, and D. W. Roberts. 1985.** Management of the Colorado potato beetle using the entomogenous fungus *Beauveria bassiana.* Am. Potato J. 62: 29–37.

**Carruthers, R. I., T. S. Larkin, and R. S. Soper. 1988.** Simulation of insect disease dynamics: an application of SERB to a rangeland ecosystem. Simulation 51: 101–109.

**Coluzzi, K. L. 2005.** Arthropod scavengers of Colorado potato beetle (*Leptinotarsa decemlineata*) cadavers. MS thesis, University of Maine, Orono, ME.

**Daoust, R. A., and R. M. Pereira. 1986.** Stability of entomopathogenic fungi *Beauveria bassiana and. Metarhizium anisopliae* on beetle-attracting tubers and cowpea foliage in Brazil. Environ. Entomol. 15: 1237–1243.

**Fargues, J. 1972.** Etude des conditions d'infection des larves de Doryphore, *Leptinotarsa decemlineata* (Say), par. *Beauveria bassiana* (Bals.) Vuill. (*Fungi imperfecti*). Entomophaga. 17: 319–337.

**Fernandez, S. 2000.** Study of conidia production and transmission of *Beauveria bassiana* (Balsamo) Vuill. in Colorado potato beetle (*Leptinotarsa decemlineata*). PhD dissertation, University of Maine, Orono, ME.

**Fraenkel, G. S., and D. L. Gunn. 1940.** The Orientation of animals. Dover Publications, New York.

**Furlong, M. J., and J. K. Pell. 2001.** Horizontal transmission of entomopathogenic fungi by the diamondback moth. Biol. Control 22: 288–299.

**Hajek, A. E., R. S. Soper, D. W. Roberts, T. E. Anderson, K. D. Biever, D. N. Ferro, R. A. Lebrun, and R. H. Storch. 1987.** Foliar applications of. *Beauveria bassiana* (Balsamo) Vuillemin for control of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae): and overview of pilot test results from the northern United States. Can. Entomol. 119: 959–974.

**Huang, H., Z. Smilowitz, M. C. Saunders, and R. Weisz. 1995.** Field selection for esfenvalerate resistance by the Colorado potato beetle. Am. Potato J. 72: 1–12.

**Inglis, G. D., Ivie, T. J., Duke, G. M., and Goettel, M. S. 2000.** Influence of rain and conidial formulation on persistence of *Beauveria bassiana* on potato leaves and Colorado potato beetle. Biol. Control 18: 55–64.

**Joergensen, H. 2000.** A model to simulate primary infection of the Colorado potato beetle (*Leptinotarsa decemlineata*) with the fungus *Beauveria bassiana.* MS thesis, University of Maine, Orono, ME.

**JMP. 2001.** Statistics and graphics guide, version 4. SAS Institute, Cary, NC.

**Kelly-Tunis, K. K., B. L. Reid, and M. Andis. 1995.** Activity of entomopathogenic fungi in free-foraging workers of *Camponotus pennsylvanicus* (Hymenoptera: Formicidae). J. Econ. Entomol. 88: 937–943.

**Kermack, W. O., and A. G. McKendrick. 1927.** Contributions to the mathematical theory of epidemics, part 1. Proc. Roy. Soc. Edin. A. 115: 700–721.

**Klinger, E. G. 2003.** Susceptibility of adult Colorado potato beetle (*Leptinotarsa decemlineata*) to the fungal entomopathogen *Beauveria bassiana.* MS thesis, University of Maine, Orono, ME.

**Kramm, K. R., D. F. West, and P. G. Rockenback. 1982.** Termite pathogens: transfer of the entomopathogen *Metarhizium anisopliae* between. *Reticulitermes sp.* termites. J. Invertebr. Pathol. 40: 1–6.

**Long, D. W., F. A. Drummond, E. Groden, and D. Donahue. 1999.** Modeling insect pathogen dynamics. Trends Entomol. 2: 55–62.

**Long, D. W., E. Groden, and F. A. Drummond. 2000a.** Horizontal transmission of *Beauveria bassiana* (Bals.) Vuill. Agri. For. Entomol. 2: 11–17.

**Long, D. W., F. A. Drummond, and E. Groden. 2000b.** Modeling *Beauveria bassiana* horizontal transmission. Agri. For. Entomol. 2: 19–32.

**Lord, J. C. 2001.** Response of the wasp *Cephalonomia tarsalis (Hymenoptera: Bethylidae) to. Beauveria bassiana* (Hyphomycetes: Moniliales) as free conidia or infection in its host, the sawtoothed grain beetle, *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). Biol. Control 21: 300–304.

**Mena-Covarrubias, J., F. A. Drummond, and D. L. Haynes. 1996.** Population dynamics of the Colorado potato beetle (Coleoptera: Chrysomelidae) on horsenettle in Michigan. Environ. Entomol. 25: 68–77.

**Olson, E. R., G. P. Dively, and J. O. Nelson. 2000.** Baseline susceptibility to imidacloprid and cross resistance patterns in Colorado potato beetle (Coleoptera:Chrysomelidae) populations. J. Econ. Entomol. 93: 447–458.

**Pimentel, D. 2005.** Environmental and economic costs of the application of pesticides primarily in the United States. Environ. Dev. Sustain. 7: 229–252.

**Slifker, J. F., and S. S. Shapiro. 1980.** The Johnson system: selection and parameter estimation. Technometrics 22: 239–246.

**SigmaPlot. 1998.** SigmaPlot 5.0 user's guide. SPSS, Chicago, IL.

**Stewart, J. G., G. C. Kennedy, and A. V. Sturz. 1997.** Incidence of insecticide resistance in populations of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera:Chrysomelidae), on Prince Edward Island. Can. Entomol. 129: 21–26.

**Tanada, Y., and H. K. Kaya. 1993.** Insect pathology. Academic, New York.

**Thomas, M. B., S. N. Wood, and C. J. Lomer. 1995.** Biological control of locusts and grasshoppers using a fungal pathogen: the importance of secondary cycling. Proc. R. Soc. Lond. B. 259: 265–270.

**Villani, M. G., S. R. Krueger, P. C. Schroeder, F. Consolie, N. H. Consolie, L. M. Preston-Wiley, and D. W. Roberts. 1994.** Soil application effects of *Metarhizium anisopliae* on Japanese Beetle (Coleoptera:Scarabaeidae) behavior and survival in turfgrass microcosms. Biol. Control 23: 502–513.

**Walstead, J. D., R. F. Anderson, and W. J. Stambaugh. 1970.** The effects of environmental conditions on two species of muscardine fungi (*Beauveria bassiana* and *Metarhizium anisopliae*). J. Inverter. Pathol. 16: 221–226.

**Watanabe, H. 1987.** The host population, pp. 71–112. *In* J. R. Fuxa and Y. Tanada (eds.), Epizootiology of insect diseases. Academic, New York.

**Zhao, J. Z., B. A. Bishop, and E. J. Grafius. 2000.** Inheritance and synergism of resistance to imidacloprid in the Colorado potato beetle (Coleoptera: Chrysomelidae). J. Econ. Entomol. 93: 1508–1514.

*Received for publication 25 November 2005; accepted 24 May 2006.*